

# Lipase-catalyzed Synthesis and Characterization of Stearic acid Dextrin Ester

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## Abstract

The process of enzymatic catalysis for the synthesis of maltodextrin ester was investigated. The single factors which affected this technology were analysed. Under the optimum conditions of 7g / L lipase, and acid/maltodextrin molar ratio of 4:1 at 65°C for 60 h, the optimum process was achieved. The as-prepared maltodextrin ester was confirmed by Fourier-transform infrared spectra (FTIR). Finally, the properties were investigated by scanning electron microscope (SEM) and Differential Scanning Calorimeter (DSC). The properties were fundamental in terms of controlling the characteristic of the final polymeric surfactants.

## Keywords

Maltodextrin; Fatty Acid; Emulsification

## Introduction

Maltodextrin, also known as water-soluble dextrin, is a product of hydrolysis between starch and sugar. The dextrose equivalent (DE), which indicates the degree of hydrolysis, is inversely proportional to molecular weight, and affects the sweetness, viscosity, solubility and colorability. The maltodextrin mainly consists of  $\alpha$  (1-4) linked D-glucose units, and the -OH groups on the number 2, 3, 5 carbon show great activity. In order to achieve the best properties of maltodextrin, -OH groups are replaced by some functional groups through chemical reactions such as oxidation, rearrangement, molecular replacement, crosslinking and esterification. The maltodextrin ester is a degeneration dextrin produced by biocatalyst which is modified by lipase. Enzymatic catalysis has been applied for several decades to the synthesis of biosurfactants based on carbohydrates. The advantages of enzymatic catalysis are mainly related to the mild conditions required, chemical selectivity of the reaction, low consumption, less byproduct, and pollution.

Maltodextrin is a hydrophilic molecule that does not have the emulsification. It can be modified by

the attachment of hydrophobic groups through a transesterification reaction with a fatty ester for emulsification. The structure of the dextrin fatty acid ester is shown in Fig. 1. Because of the good emulsification, lubrication, solubilization, foaming, defoaming, innocuity, odorless, non-irritating, non-irritating and other effects, maltodextrin esters can be used in ice cream, soup, milk beverages, tooth paste, lotions, and lipsticks. In this study, maltodextrin ester was synthesized by lipase catalyzed reaction of maltodextrin and stearic acid in the presence of immobilized lipase in t-butyl alcohol. Reaction pathway of dextrin ester was shown in Fig. 2. Influence of experimental conditions, such as lipase concentration, mass ratio of acid/ maltodextrin, reaction time, and reaction temperature on conversion, was investigated. The molecular structure of enzymatic synthesized maltodextrin ester was characterized by Fourier-transform infrared spectroscopy (FTIR). The properties were investigated by scanning electron microscope (SEM) and Differential Scanning Calorimeter (DSC).

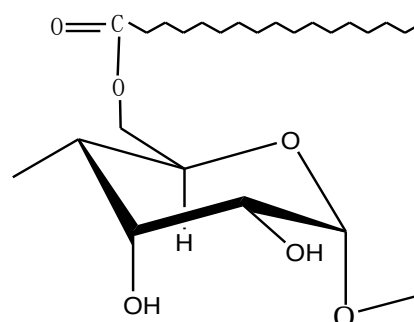


FIG. 1 THE STRUCTURE OF THE DEXTRIN FATTY ACID ESTER

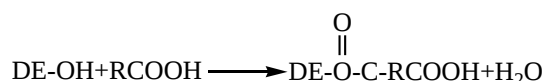


FIG. 2 LIPASE-CATALYZED SYNTHESIS PATHWAY OF DEXTRIN

## Materials and Methods

### Materials

Lipase Novozym 435, immobilized on a macroporous

exchange resin, was purchased from Beijing Gao Ruisen, Technology Co. Ltd. (Beijing, China). According to the specification sheets, the activities were 12,200 U/g and used directly without any pretreatment. Stearic acid was purchased from Tianjin Bodi Chemical Co. Ltd. (Tianjin, China). Dextrin was bought from Yuan Hang Co. Ltd. (Jiangsu, China) T-butyl alcohol was dried over molecular sieves for 24 h prior to use.

### Methods

The reaction mixture used for reaction contained 100 mL t-butyl alcohol, 3 g of maltose, stearic acid (1.5-21 g, the molar ratios of acid/maltodextrin ranging from 2:1 to 6:1), and Novozym435 (0.4-0.6g). To improve the conversion, 20 g/L of molecular sieve (3Å) was added to absorb water generated from the reaction mixture during esterification. The enzymatic reaction was under stirring in a water bath at 60-70°C for 48-72 h. Control experiment without enzyme was carried out with the same procedure.

The extent of dextran modification (%) was determined by DS (degree of substitution).

### Analysis

Degree of substitution (DS) was measured by titration. 2.00 g dry weight of the maltodextrin ester was suspended in 50 mL distilled water, mixing 20.0 mL of 0.250 M NaOH and stirred for 2 h. Then the solution was titrated with 0.100 M HCl solution, using phenolphthalein as an indicator control experiment without dextrin ester was carried out with the same condition. The DS was calculated using the following equations:

$$DS = \frac{162\omega}{1000m - 266\omega} = \frac{162c(V_0 - V_1)}{1000m - 266c(V_0 - V_1)} \times 100\%$$

Where  $\omega$  is mass fraction of Stearyl acyl, %;

162 is molar mass of anhydride Glucose unit (AGU)  $\text{g} \cdot \text{mol}^{-1}$ ;

266 is Relative molecular mass of stearyl acyl  $\text{CH}_3(\text{CH}_2)_{16}\text{CO}$ ;

C is amount-of-substance concentration of HCl,  $\text{mol} \cdot \text{L}^{-1}$ ;

M is the dry weight (g) of the sample;

$V_0$  is the titration volume of NaOH (mL) for control;

$V_1$  is the titration volume of NaOH (mL) for sample.

### Fourier Transforms Infrared (FT-IR) Spectroscopy

The FT-IR spectra of the maltodextrin and maltodextrin ester were recorded in an IR spectrometer, using potassium bromide (KBr) discs prepared from powdered samples mixed with dry KBr in the ratio 1:150.

### SEM

SEM micrographs were taken using scanning electron microscopy of SEMX-300 (Phillip FEI QUANTA 200Company) with a secondary electron beam with acceleration gradient of 20 eV.

### Differential Scanning Calorimetry (DSC)

Measurements were performed on 20 mg samples sealed in aluminum pan and heated in the range of 20–200°C with the rate of 10°C/min. Empty capsule served as a reference. Self-assembled in Department of Physics was used. Measurements were conducted in triplicates.

## Results and Discussion

### Influence of the Molar Ratios of Acid/ Maltodextrin on DS

The Influence of the molar ratios of acid/maltodextrin was investigated. In this study the reactions were performed at the molar ratios of acid/ maltodextrin of 1:2 to 6:1 at the condition of 0.7g Novozym435 and 20 g/L molecular sieve under stirring in a water bath at 65 °C for 60 h. As can be seen from fig.3, the conversion increased with the molar ratios increasing, but after a certain molar ratio of 4:1, it decreased. The appropriate concentration of compound is important in lipase-catalyzed acylation since this reaction takes place via the formation of an acyl-enzyme intermediate. The lower substrate concentration is, the smaller probability of enzyme activity sites gets, so it is difficult to catalyse reactions. But the higher concentration will destroy the balance of etherification, and then the reaction is hindered.

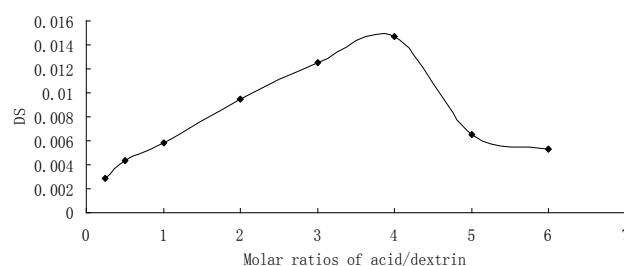


FIG. 3 EFFECT OF MOLAR RATIO OF ACID/DEXTRIN ON DS

### Influence of Enzyme Concentration on DS

In this study, the influence of enzyme concentration was studied by varying enzyme from 3 to 8g/L at the condition of the molar ratios of acid/maltodextrin of 4 and 20 g/L molecular sieve under stirring in a water bath at 65°C for 60 h. The result is revealed in Fig.4, and the conversion is increasing while the enzyme concentration increases, but after a certain concentration

of 8g/L, the conversion tends to steady.

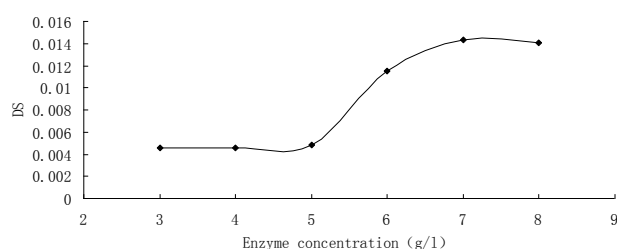


FIG. 4 EFFECT OF ENZYME CONCENTRATION ON DS

### *Influence of Reaction Time on DS*

In this study, the influence of enzyme concentration was studied by varying reaction time from 12 to 90 h at the condition of the molar ratios of acid/ maltodextrin of 4, 0.7 g Novozym435 and 20 g/L molecular sieve under stirring in a water bath at 65°C. The result can be seen from Fig 5, in which the conversion increased with the increasing reaction time. But after 60 h, no further increase in DS occurred on further heating. In the initial state, due to the low solubility of the substrate, the substrate cannot combine with the enzyme quickly, so it shows low reaction rate. As the reaction proceeded, water was absorbed by molecular sieve, so the balance of the reaction was disturbed, which brought a positive response.

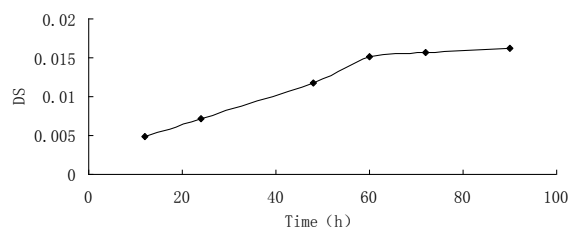


FIG. 5 EFFECT OF REACTION TIME ON DS

### *Influence of Reaction Temperature on DS*

In this study, the influence of reaction temperature was studied by varying reaction temperature from 55°C to 60°C at the condition of the molar ratios of acid/maltodextrin of 4, 0.7 g Novozym435 and 20 g/L molecular sieve under stirring in a water bath at 60 h. The effect of temperature is shown in Fig. 6. The boiling point of the solvent, the solubility of substrate and the lipase activity are influenced by the temperature. So, the conversion increased with the increasing temperature when the system temperature is below 60°C. A solvent will dissolve more solute with a higher temperature. It is good to reaction substrate for diffusion bonding with each other as well as the excitation of the enzyme activity. It reached equilibration value at 60-70°C. When temperature reached higher than 70 °C , the DS decreased

significantly, resulting from thermal deactivation of enzyme. Low or high temperatures will result in poor conversion because of the obviously relatively low enzyme activity.

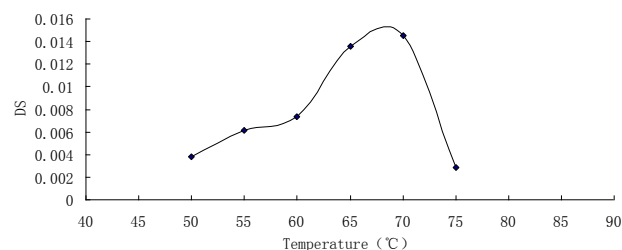


FIG. 6 EFFECT OF REACTION TEMPERATURE ON DS

### *FTIR Analysis*

The revolution of the FTIR spectra of maltodextrin and maltodextrin ester with scanning range from 4000 to 500  $\text{cm}^{-1}$  was shown in Fig 7, in the spectrum of pure sample, a strong broad band between 980  $\text{cm}^{-1}$  and 1200  $\text{cm}^{-1}$  was the most characteristic band for a polysaccharide. It was attributed to the C-O stretching vibrations. A new absorption band at 1,726  $\text{cm}^{-1}$  was assigned to esterbond appeared in maltodextrin ester. At the same time, because part of the maltodextrin-OH is esterified to the carbonyl, O-H stretching band at 3400  $\text{cm}^{-1}$  in the maltodextrin ester decreased obviously in intensity following the esterification reaction. Furthermore, the bank of fatty group at 2849.95  $\text{cm}^{-1}$  in spectra also confirmed that the dextrin ester was successful achieved by esterification.

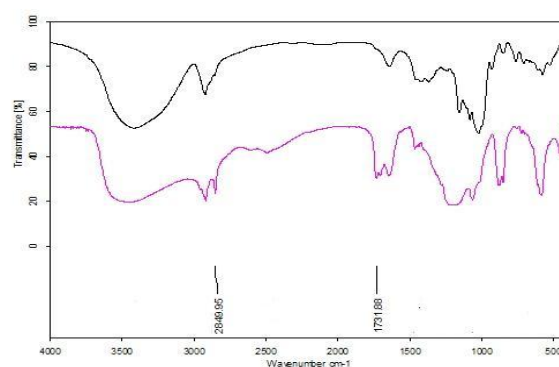
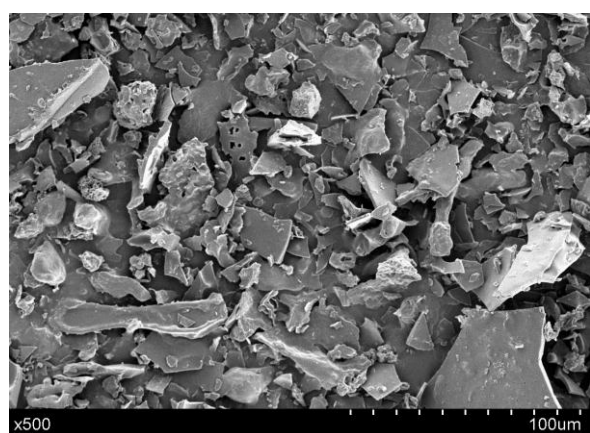


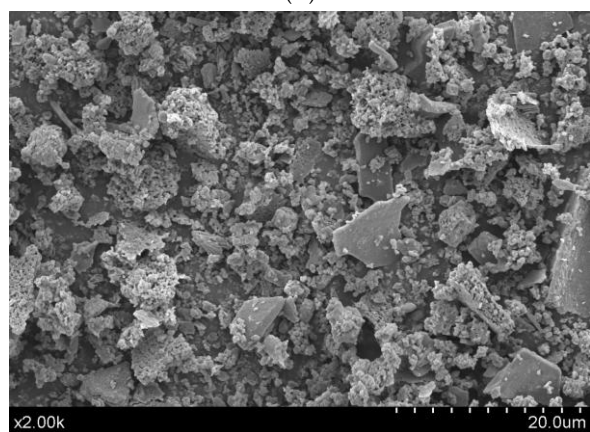
FIG. 7 FOURIER-TRANSFORM INFRARED SPECTRA OF DEXTRIN AND DEXTRIN ESTER.

### *SEM Analysis*

As shown in Fig.8, appearance of the reaction products under scanning electron microscope (SEM) revealed that the granularity of the products was retained but granules suffered essential damage. Disruption of maltodextrin granules became more apparent with the reaction of the esterification. The SEM micrographs exhibited that granules were agglutinated.



(A)



(B)

FIG. 8 SEM PHOTOGRAPHS OF MALTODEXTRIN (A) AND MALTODEXTRIN ESTER (B)

### DSC

DSC was used to measure the occurrence of exothermal or endothermal changes with increasing in temperature. The DSC profile of maltodextrin and maltodextrin ester showed that there was a strong endothermal peak around 50 to 100°C, corresponding to the evaporation of residual free water in the samples. However, in this area, the width and height of maltodextrin ester were significantly smaller than those of the maltodextrin. It showed that the water binding capacity of dextrin ester was weakened. Because of a large number of hydroxyl, the dextrin showed strong affinity of water. But the hydroxyls in the dextrin ester were replaced by carbonyl, which enhanced the hydrophobic and diminished the hydration. At the same time, the glass transition temperature of dextrin was lower than that of the dextrin. It was due to the introduction of the ester groups which caused the expansion and breaking of the dextrin. So the Molecular tension decreased and the molecular mobility increased.

The melting temperature of the maltodextrin and maltodextrin ester were 62, 61°C and 52.74°C. The

enthalpy of the dextrin and dextrin ester were 25.86 J/g and 2.76 J/g, respectively. The enthalpy of the dextrin decreased significantly, which further demonstrated the stearic acid brought activation in the reaction. In all, the dextrin showed a better thermoplastic.

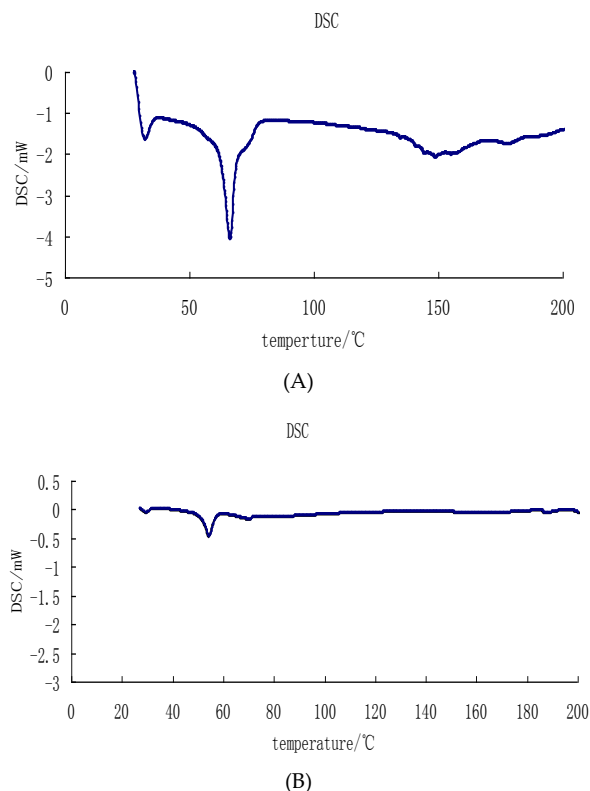


FIG. 9 DSC OF MALTODEXTRIN (A) AND MALTODEXTRIN ESTER (B)

### Conclusion

Dextrin ester was prepared by reaction of maltodextrin with Stearic acid in organic solvent. Maltodextrin had the disadvantages in terms of hydrophobic property and emulsifiability, so Lipase-catalyzed synthesis was a method to overcome these shortcomings. The highest DS for the synthesis of maltodextrin ester was obtained under optimized reaction conditions of 7 g/L lipase from Novozym 435, and acid/maltodextrin molar ratio of 4:1 at 65°C for 60 h. This simple enzymatic synthetic methodology provided an environmentally friendly method of preparing maltodextrin by minimizing energy consumption, solvent, toxicity and amount of catalyst needed, increasing reaction efficiency, and minimizing byproducts. Maltodextrin ester was confirmed by FTIR, SEM and DSC.

### ACKNOWLEDGEMENTS

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